THE USE OF POLYETHYLENE VESSELS FOR PREPARING AND TRANSPORTING TISSUE CULTURES

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[Following is the translation of an article by G. A. Bashmakov and V. N. Tarasov, Virus Scientific-Research Laboratory and the Microbiology Department of the S. M. Kirov Order of Lenin Military-Medical Academy, published in the Russian-language periodical Voprosy Virusologii (Problems of Virology) 1963, 8(5), pages 628--629. It was submitted on 1 Feb 1963. Translation performed by Sp/7 Charles T. Ostertag Jr.]

At the present time all the virological laboratories use only glass vessels, primarily vessels made from neutral glass, for the cultivation of cellular strains. Meanwhile such vessels have a number of deficiencies.

We considered it advantageous to test certain types of vessels made from an unbreakable material for the incubation of tissue cultures and the storage of nutrient media.

Materials and Methods

In the tests in place of glass separating flasks we used 250 ml polyethylene quadrangular flasks, and in place of glass bottles for storing and transporting nutrient solutions and media we used 250 and 500 ml polyethylene bottles. * In a number of tests the cellular monolayer was incubated in foreign made celloidin test tubes. Test tubes and separating flasks made out of neutral glass were used as a control.

For setting up the experiments we used monolayer transplanted cultures from the renal epithelium of monkeys (strain MKS), the amniotic membranes of human placenta, human liver cells (Chang strain), human bone marrow (Detroit-6 strain), and also permanently transplanted HeLa and HEp-2 cancerous cells. Along with the pure cellular lines we also incubated a mixed culture from HEp-2 and MKS cells.

Before use the clean flasks made out of polyethylene and the test tubes made from celloidin were sterilized with 96% alcohol (one hour exposure time). For removing the residue of the alcohol it was necessary to wash the vessels 2-3 times with sterile Hank's solution, after which they were ready for use.

The method of incubating monolayer tissue cultures of transplanted cells in polyethylene flasks is no different from the generally accepted method.

* The vessels made out of synthetic material were made by the "Komsomolskaya Pravda" Plastics Plant in Leningrad.

The cellular suspension in the appropriate nutrient medium is placed into a 20--25 ml flask (cell concentration 250,000 in 1 ml). For obtaining these tissue cultures we used a growth medium of the following composition: 80% medium No 199, 20% donor serum, based on 250 units of antibiotics (penicillin and streptomycin) in 1 ml.

After placing the cellular suspension into the flask the inner cork must be replaced by a rubber one which fits closely against the neck and guarantees a ratio of the gaseous mixture which is optimum for the growth of cells. The incubation was carried out at 37°.

Upon the appearance of a solid cellular layer the incubating cells are removed with trypsin or Versene. For this initially the nutrient phase was drawn off and then 10 ml of an 0.25% solution of trypsin or an 0.02% solution of Versene is placed into the flask. At 37° the complete stripping of the cells from the wall of the flask took place in 5--20 minutes.

A volume of 1.5 ml (concentration of cells 80,000 in 1 ml) of a cellular suspension was placed in the celloidin test tubes for cultivation.

Results

In the course of conducting the experiments the possibility was studied for the growth and multiplication of cells on the wall of polyethylene flasks, celloidin test tubes, and also on glass test tubes and separating flasks. The comparative data of cultivating stable cellular strains in these vessels are presented in the table.

It follows from the table that the fixation of cells of the majority of stable transplanted strains (MKS, HEp-2, HeLa), both in pure and in mixed cultures, to the surface of the polyethylene flask, the celloidin test tubes and the glass vessels (separating flasks, test tubes) and growth begin practically at the same time -- in the first or second 24 hour period following the introduction of the cellular suspension into the vessel. An expressed monolayer of these cells is detected in the polyethylene flask and the celloidin test tube on the 4--6th day of cultivation, that is, in the same periods as in the glass vessels.

Amniotic cells become attached to polyethylene and celloidin less effectively and grow slower than on glass, forming a monolayer culture only on the 8--10th day of incubation. The types of synthetic vessels used turned out to be unsuitable for the incubation of cultures of transplanted Chang and Detroit-6 cells.

The concept that vessels made of polyethylene, due to their weak transparency, did not permit the carrying out of microscopic investigations of tissue cultures turned out to be an error. The limited transparency of the walls of polyethylene flasks not only did not hinder the microscopic control

over the growth of the cells, but it was also possible to record the picture being observed with the help of microphotographing.

In the second series of experiments we checked the feasibility of using polyethylene bottles (250 and 500 ml) for the storing of liquids, nutrient solutions and media (Hank's solution, medium No 199, lactalbumin hydrolyzate, Earle's medium and several others) which are generally used in the practice of tissue culture. These solutions were stored in polyethylene vessels at room temperature and under the conditions of a household refrigerator for a period of a month, after which they were used in the preparation of tissue cultures.

These experiments showed that the biological activity and the pH of nutrient solutions and media do not change when stored under these conditions. Qualitatively they were no different from solutions kept in glass vessels.

The previously used polyethylene vessels and celloidin test tubes were processed with the help of detergents which guaranteed the complete stripping of cells from the surface of the flasks and test tubes and the qualitative washing of the vessels. As the detergent it was convenient to use the commercially available powder "Progress" (composition: 30% sulfanol, 7--8% tripolyphosphate, 55% sodium sulfate, 5--8% admixtures; pH 7--8).

The flasks and test tubes were completely submerged in an 0.5% solution of the detergent, after which the solution was heated up to 60--70° (30 minute exposure time). Subsequently the vessels were carefully rinsed in warm running water and rinsed several times with distilled water.

Growth of transplanted cells and the formation of monolayer tissue cultures in polyethylene flasks, celloidin test tubes and glass vessels.

Strain of cells	Polyethylene flasks and celloidin test tubes		Glass test tubes and separating flasks	
	Beginning of cell growth	Formation of monolayer culture	Beginning of cell growth	Formation of monolayer culture
	in days			
MKS	1--2	5--6	1--2	5--6
Amniotic cells	4	8--10	2--3	5--6
Chang	Not attached	No growth	3	7--8
Detroit-6	"	" "	2--3	7
HeLa	1--2	4--5	1--2	4
HEp-2	1--2	4--5	1--2	4--5
Mixed culture HEp-2 and MKS	1--2	5--6	1--2	5--6